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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING ELASTASE

	V	IV	III	II	I	
Residue #	350 +	•	•	•	•	360
AAT:	ALA - MET - PHE - LEU - GLU - ALA - ILE - PRO - MET - SER - ILE -					
SPAAT:	MET - PHE - LEU - GLU - ALA - ILE - PRO - MET - SER - ILE -					
						370
AAT:	PRO - PRO - GLU - VAL - LYS - PHE - ASN - LYS - PRO - PHE -					
SPAAT:	PRO - PRO - GLU - VAL - LYS - PHE - ASN - LYS - PRO - PHE -					
						380
AAT:	VAL - PHE - LEU - MET - ILE - GLU - GLN - ASN - THR - LYS -					
SPAAT:	VAL - PHE - LEU - MET - ILE - GLU - GLN - ASN - (THR) - LYS -					
						390
AAT:	SER - PRO - LEU - PHE - MET - GLY - LYS - VAL - VAL - ASN -					
SPAAT:	SER - PRO - LEU - PHE - MET - <u>LEU</u> - LYS - VAL - VAL - <u>SER</u> -					
						394
AAT:	PRO - THR - GLN - LYS - COOH					
SPAAT:						

(57) Abstract

This invention provides a compound comprising: (1) a polypeptide moiety having a) an identifying number of amino acids for SPAAT, b) an elastase binding activity; and (2) an extracellular matrix protein bound to the polypeptide moiety. Also provided is a method of inhibiting an elastase comprising contacting the elastase with a polypeptide moiety having: (1) an identifying number of amino acids for SPAAT; (2) a collagen binding activity; and (3) elastase binding activity.

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COMPOSITIONS AND METHODS FOR INHIBITING ELASTASE

Throughout this application various publications are referenced. The citations are provided immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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TECHNICAL FIELD
BACKGROUND ART

α 1 Antitrypsin (AAT) is a 52 kDa plasma serine protease inhibitor. Its normal plasma concentration ranges from 150 to 350 mg/dl (Brantly et al., 1988), although it behaves as an acute phase reactant increasing 3-4-fold during host response to inflammation and/or tissue injury such as with pregnancy, acute infection, tumors, estrogen, and typhoid vaccine (Kushner, 1988; Schreiber, 1987). AAT is capable of inhibiting a variety of proteases including trypsin, chymotrypsin, plasmin, thrombin, kallikrein, factor Xa, plasminogen and cathepsin G (Carrel et al., 1986; Laurell & Jeppson 1975; Travis & Salvesen, 1983), but its main physiological role is the inhibition of neutrophil elastase. Neutrophil elastase is not only capable of attacking elastin but may also cleave other connective tissue proteins, such as type I, III, and IV collagens, the protein portion of proteoglycans, and laminin (Bieth, 1986). AAT, however, prevents such degradation by forming a tightly bound 1:1 enzyme:inhibitor complex resulting in the slow proteolytic cleavage of the reactive center of the inhibitor between MET-358 and SER-359 of AAT.

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The reactive center of human AAT is contained within an exposed peptide from (ALA-350) to (SER-359) in a stressed loop configuration (Carrell, 1986; Bruch et al.,

1988), which may be comparable to the bait region of α -2-macroglobulin (S-Jensen, 1987). Every serine proteinase so far examined, including bacterial, plant, and reptilian as well as mammalian enzymes, has been reported to disrupt
5 bonds within this loop of the native inhibitor. Cathepsin L (Johnson et al., 1986) and Serratia marcescens metalloproteinase (Virca et al., 1982), for example, have been reported to cleave the peptide bond between MET-358 and SER-359, while Pseudomonas aeruginosa (Moriyama et
10 al., 1984), macrophage elastase (Banda et al., 1985) and PMN collagenase (Knäuper et al., 1990) have been reported to cleave one amino acid residue N-terminal at the peptide bond between PRO-357 and MET-358. Cathepsin L (Johnson et al., 1986) and Staphylococcus aureus cysteine and serine
15 proteinase (Potempa et al., 1986) have also been reported to cleave the peptide bond between GLU-354 and ALA-355, while Staphylococcus aureus metalloproteinase (Potempa et al., 1986), secreted PMN metalloproteinase (Desochers and Weiss, 1988, Vissers et al., 1988), and PMN collagenase
20 (Knäuper et al., 1990) have been reported to cleave two amino acids N-terminal at the peptide bond between PHE-352 and LEU-353. In addition, Crotalus adamanteus (The Eastern Diamondback Rattlesnake) venom proteinase II has been reported to cleave the peptide bond between ALA-350
25 and MET-351. While cleavage of the inhibitor does not effect the inhibition of the bound AAT, the general consensus is that such fluid-phase cleavage inactivates the inhibitor from inhibiting other proteases. (Michaelis et al., 1990; Knäuper et al., 1990). There currently is
30 no known function of the smaller cleaved peptide, although it may bind hepatoma and monocyte receptors (Perlmutter et al., 1990).

Kress et al., 1989, report that venom proteinase
35 II from the Eastern Diamondback Rattlesnake cleaves AAT in-vitro between ALA-350 and MET-351. Additionally, Kress et al., disclosed that one of the fragments produced in

the cleavage has the NH₂ terminal sequence: Met-Phe-Leu-Glu-Ala-Ile-Pro-Met-Ser-Ile-Pro-Pro-Gln-Val-Lys-Phe-Asn. Kress et al., disclose no activity for the fragment and disclose that the fragment does not inhibit the cleavage of trypsin by an elastase.

This invention provides the discovery that a 44-residue, C-terminal fragment of AAT (hereinafter designated "SPAAT") exists in human subjects. This fragment appears to represent the same fragment disclosed by Kress et al. when AAT was cleaved by snake venom proteinase II. However, despite the absence of activity being attributed to any small fragment of cleaved AAT in the literature and the finding in Kress et al. of no activity associated with the fragment, this invention provides the surprising discovery that SPAAT is in fact a potent inhibitor of elastase. Additionally, the invention provides the completely unexpected discovery that SPAAT, when bound by an extracellular matrix protein such as a collagen, inhibits elastase to a much greater degree than SPAAT alone.

Since SPAAT, or equivalent polypeptides can be synthesized, the invention provides a much needed effective and inexpensive method to treat conditions such as emphysema and respiratory distress syndrome. Additionally, because of the discovery that the unique association of SPAAT with the extracellular matrix (ECM) greatly increases the half-life, SPAAT can be administered much less frequently than AAT. This leads to less expensive administration and greater quality of life for the patient.

Finally, since the invention demonstrates that in vivo SPAAT is bound or deposited on biologically susceptible ECM proteins, such as elastin or collagen,

SPAAT can be used in protectng these proteins from the inappropriate attack of enzymes like HNE.

DISCLOSURE OF INVENTION

This invention provides a compound comprising:

- 5 (1) a polypeptide moiety having
- a) an identifying number of amino acids for SPAAT,
 - b) an elastase binding activity; and
- (2) an extracellular matrix protein bound to the polypeptide moiety.

10 Also provided is a method of inhibiting an elastase comprising contacting the elastase with a polypeptide moiety having:

- 15 (1) an identifying number of amino acids for SPAAT;
- (2) a collagen binding activity; and
 - (3) elastase binding activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a comparison of C-terminal amino acid sequence of AAT with that of the sequenced portion of SPAAT isolated from human placenta. Residue numbering represents the position of each amino acid within the intact AAT sequence. The two differing residues, LEU-386 and SER-390, are underlined. Arrows (+) indicate the cleavage sites of various proteolytic enzymes on complexing with AAT: I = Cathepsin L, Serretta marcescens metalloproteinase. II = Pseudomonas aeruginosa, macrophage elastase, PMN collagenase. III = Cathepsin L, Staphylococcus aureus cysteine and serine proteinase. IV = Staphylococcus aureus metalloproteinase, secreted PMN metalloproteinase, PMN collagenase. V = Crotialus adamanteus venom proteinase II.

Figure 2 shows the inhibition of various serine proteases by SPAAT. Chymotrypsin (O); HNE (square); Pancreatic elastase (triangle); Trypsin (*). For comparison, the inhibition of HNE by AAT (---) under these assay conditions was also determined.

Figure 3 shows the binding of AAT and SPAAT to DFP-treated human neutrophil: A. Elastase. B. Cathepsin G.

BEST MODE OF CARRYING OUT THE INVENTION

The data presented below demonstrates that the C-terminal 44 amino acid reactive center containing
5 fragment of AAT, SPAAT, can be tissue bound and play an important physiological role in the protection of ECM proteins from the inappropriate attack of HNE. SPAAT was isolated and sequenced (Figure 1) from human placenta that had been extensively extracted. This binding,
10 furthermore, appears to be specific as no human serum albumin (HSA), another plasma protein found in 10X the concentration of AAT, was detected by ELISA (Table I).

Thus, the invention provides a composition
15 comprising:

- (1) a polypeptide moiety having
 - a) an identifying number of amino acids for SPAAT,
 - b) an elastase binding activity; and
- 20 (2) an extracellular matrix protein bound to the polypeptide moiety.

While Figure 1 only discloses amino acids through position 390, the composition of this invention includes -PRO-THR-GLN-LYS, the amino acids at positions 391-394.
25 Additionally, as noted elsewhere, positions 390 and 386 likely represent sequencing errors and should correspond to the sequence for AAT at those positions. In this regard, sequences corresponding to amino acids 351 through 394 of AAT were synthesized and tested as set forth in the
30 Example. Identical results were obtained for the synthesized peptide as for the placenta derived peptide set forth in the Example. Thus, the sequence for SPAAT set forth in Figure 1 and the amino acid sequence for residue 351-394 of AAT as used herein are synonymous.

35

Given the sequence of SPAAT set forth in Figure 1, one could follow standard methods to make additions,

substitutions and deletions to vary the sequence of SPAAT. These variations could be tested using the methods set forth in the example to determine elastase binding activity. Thus, "SPAAT" means a moiety which has the
5 essential amino-acids of SPAAT for elastase and collagen binding activity.

As set forth in the examples, SPAAT binds an extracellular matrix protein Type I collagen to have an
10 increased inhibitory capacity. Other extracellular matrix proteins would be expected to likewise increase activity by inducing a conformational change in the peptide. The reason for SPAAT's binding to collagen is likely due to hydrophobic interactions. SPAAT is a hydrophobic peptide
15 and collagen has clusters of hydrophobic regions. Thus, since other extracellular matrix proteins likewise contain hydrophobic regions, especially elastin, they would be expected to have the same interactions. At any rate, such activity can be routinely tested based on the teaching in
20 the example. Thus, only those extracellular matrix proteins which actually bind the polypeptide are included within the scope of the composition claim. Elastin is another example of an extracellular matrix protein which would be expected to bind the polypeptide moiety.
25 Additionally, the compositions of the invention can be combined with a pharmaceutically acceptable carrier for administration.

30 The invention also provides a kit comprising:
(1) a polypeptide moiety having
a) an identifying number of amino acids for SPAAT,
b) an elastase binding activity; and
35 (2) an extracellular matrix protein. The extracellular matrix protein can be a collagen, especially Type I collagen.

The invention still further provides a method of inhibiting an elastase comprising contacting the elastase with a polypeptide moiety having:

- 5 (1) an identifying number of amino acids for SPAAT;
- (2) a collagen binding activity; and
- (3) elastase binding activity.

This method applies to any elastase which is bound by the moiety, for example neutrophil elastase. One would expect
10 other elastases to be inhibited by the polypeptide moiety due to similarities of cleavage mechanisms and substrate specificities of the elastases. In one embodiment, the polypeptide moiety of the method has the identical amino acid sequence of SPAAT.

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Additionally, the method of inhibiting can be practiced by contacting the polypeptide moiety with an activity-enhancing extracellular matrix protein, for example, type I collagen or elastin, prior to contacting
20 the elastase. Such a method can increase the activity of the polypeptide moiety leading to greater inhibitor activity.

Finally, the invention provides a method of
25 treating an aberrant condition associated with proteolysis by neutrophil elastase in a subject comprising administering to the subject polypeptide moiety having:

- (1) an identifying number of amino acids for SPAAT shown;
- 30 (2) a collagen binding activity; and
- (3) neutrophil elastase binding activity.

Many aberrant conditions can be treated by this method. The aberrant conditions include pulmonary emphysema and adult respiratory distress syndrome.

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EXAMPLE

The following EXAMPLE, inter alia, describes the isolation of SPAAT. It should be recognized that SPAAT, given the sequence described herein, could be readily synthesized or recombinantly produced.

Methods

All compounds listed below are commonly used and available and any abbreviation utilized is readily known to the skilled artisan.

Isolation of SPAAT. All operations were performed at 4°C and all extraction buffers contained 25mM EDTA, 5mM benzamidine, 1mM PMSF, 1mM NEM to minimize protein degradation during processing. After removal of membranes, the human placenta tissue was minced with scalpels and washed extensively with 1M NaCl, 50mM Tris, pH 7.5 to remove blood. The tissue was next extracted with several washes of 8M urea, 50 mM Tris, pH 7.8. The residue was then extracted with 2 volumes of 8M urea, 50 mM Tris, pH 7.8 containing 1% 2-ME. The supernatant was dialyzed extensively against 0.1 M ammonium bicarbonate, 50 mM Tris, pH 8.0, centrifuged to remove insoluble material, and gently stirred overnight with DEAE Trisacryl which had been equilibrated in the same buffer. The DEAE was recovered by low speed centrifugation and washed for 10 min with an equal volume of bicarbonate buffer. After low speed centrifugation, the proteins of interest were eluted by stirring for 3 hrs in the same bicarbonate buffer containing 0.5 M NaCl. The DEAE was removed by low speed centrifugation and the supernatant further clarified by ultracentrifugation. The solution was then dialyzed against distilled water and lyophilized to dryness. A 50 mg aliquot of the preparation was resuspended in 5 ml of 5.0 M urea, 0.1 M Tris, pH 8.5 containing 0.2 M DTT for 5

hrs, then dialyzed against a large excess of 60 mM sodium acetate, pH 4.85. The material precipitating during dialysis was removed by centrifugation. This precipitate was similarly redissolved in the above urea-DTT solution and dialyzed versus the acetate buffer two additional times. The resultant pooled supernatants were subjected to ELISA and amino acid analysis to determine yield as well as amino acid sequence analysis.

10 ELISA. Plastic microtiter wells were coated overnight at 4°C with 100 µls of antigens appropriately diluted in PBS. Plates were washed 3x with approximately 300 µls PBS/well. Any remaining reactive sites were blocked by adding 200 µls 1% BSA in PBS/well and incubating for 1 hr at 37°C. The plates were again washed 3x with approximately 300 µls PBS/well. The rabbit anti-human AAT and HSA polyclonal antibodies were diluted 1/40,000 with PBS containing 0.05% Tween-20. 50 µls of this diluted primary antibody was added/appropriate well. The plate was again incubated for 1 hr at 37°C, then washed 3x with approximately 300 µls PBS/well. 50 µls of an appropriately diluted secondary antibody (goat anti-rabbit IgG diluted 1/16,000) was added/well. The plates were again incubated for 1 hr at 37°C, then washed 5x with approximately 300 µls PBS/well. 50 µls of color developing ortho-phenylenediamine (OPD) solution was added/well. The plates were incubated for 30 min at 37°C. The reaction was finally stopped by the addition of 50 µls/well of 4.5 M sulfuric acid and read at 492 nm.

30

An ELISA was also developed to quantitate the potential binding affinity of various antigens for AAT and/or SPAAT. These ELISAs were done as outlined above, except (1) enzyme antigens (HNE, cathepsin G) were inactivated by DFP treatment prior to the ELISA to minimize potential artifacts due to the cleavage of detecting antibodies, (2) wells were coated with antigen

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solutions at approximately 40 µg/ml, and (3) after blocking wells were incubated with 10 µg/ml AAT or SPAAT in PBS for 2 1/2 hrs at 37°C. Control wells were incubated with PBS alone. The binding of exogenous AAT and SPAAT to specific antigens was corrected for the non-specific binding of AAT and/or SPAAT to BSA.

Protein Sequencing. Edman degradations were performed in a Beckman Model 890M sequencer (Bhown & Bennett, 1985). Approximately 200 pmol of the peptide was used for sequencing. Two different placental SPAAT preparation were sequenced with identical results. Repetitive yields were generally between 96 and 99%. PTH amino acids were identified by HPLC as described by Bhown & Bennett (1985).

Enzyme Assays. Serine protease assays were performed using p-nitroanalide amide substrates. Trypsin activity was assayed by using Benzyl-PHE-VAL-ARG-PNA (0.5 mg/ml). Chymotrypsin activity was assayed by using SUC-(ALA)₂-PRO-PHE-PNA (10 mg/ml in DMSO). Elastase activity was assayed by using SUC-(ALA)₂-PNA (10 mg/ml in DMSO). The dilution of each of these enzymes required to produce a change in absorbance of approximately 0.4 after a 15 min incubation at 37°C was initially determined. The complete reaction mixture contained 10 µls of this enzyme dilution plus 100 µls of the appropriate above substrate and 0-50 uls of the indicated concentration of inhibitor or TBS to maintain constant volume. Trypsin, chymotrypsin, and HNE assays were incubated at 37°C for 15 min, while pancreatic elastase assays were incubated at 37°C for 1.5 min. The reaction was terminated by the addition of a sufficient quantity of ice cold TBS to bring each assay up to a final volume of 1 ml. The absorbance of the p-nitroaniline produced was measured against a distilled water blank at 410 nm on a Hitachi model 100-40 spectrophotometer. Percent inhibition was calculated as: $100 - [(A_1E + I / A_1E) \times 100]$.

Results

Extraction. As summarized in Table I for a typical preparation, milligram quantities of antigenically detectable AAT (or its cleavage fragment(s) including SPAAT) were recovered at each extraction step. In order to establish that the tissue binding of SPAAT was specific, we compared the amount of AAT reactivity in the extracts to that of another serum protein, HSA, which is found at 10 times the concentration of AAT in the plasma. As indicated in Table I, no HSA was detected in any of these fractions. Interestingly, even after this extensive extraction procedure some AAT antigenic activity remained associated with the acid insoluble precipitate. When this pellet was solubilized in 5.0 M urea, 0.1 M Tris, pH 8.5 containing 0.2 M DTT and run on a molecular sieve column (Superose 6, Pharmacia), two peaks of AAT antigenic activity were recovered: a high molecular weight peak and a low molecular weight peak (data not shown) suggesting that some SPAAT might remain aggregated and/or be bound to one or more high molecular weight "carrier" protein(s).

Biochemical Characterization of SPAAT. When the DEAE-bound material was dissolved in 5M urea, 0.1M Tris, pH 8.5 containing 0.2 M DTT and dialyzed against 60 mM sodium acetate buffer, pH 4.85, most of the protein appeared to be insoluble and precipitated. A small amount of protein (usually 10% or less) remained soluble. This supernatant was dialyzed, lyophilized, weighed, and resuspended in 50% acetic acid. An aliquot equivalent to approximately 200 pmoles was then subjected to amino acid sequence analysis. Its 40 residue N-terminal sequence is presented in Figure 2. A computer comparison with the sequence of known proteins revealed that 38 of these 40 residues were identical to the C-terminal region of AAT beginning at MET-351, 7 amino acids N-terminal to the reactive center MET-358 (Long et al., 1984). The two differing residues,

LEU-386 and SER-390, occurred near the C-terminus of the peptide when the sequence was becoming difficult to decipher and likely represent sequencing errors. Moreover, it is apparent that SPAAT is a 44 residue protein beginning at MET-351 and ending with LYS at 394 consistent with the COOH terminal sequence of AAT.

Potential Physiological Significance

10 Inhibition of Enzyme Activity. Preliminary enzyme kinetic experiments presented in Figure 2 indicate that our desalted SPAAT preparations preferentially inhibit chymotrypsin > HNE > pancreatic elastase, while having no effect on trypsin. Importantly, these SPAAT preparations
15 inhibited HNE activity almost as well as native AAT. Preliminary calculations of the inhibitor concentrations required to totally inhibit chymotrypsin enzymatic activity indicate that SPAAT is a potent ($K_I \sim 1/10 K_m$), competitive inhibitor of chymotrypsin.

20 Mode of Inhibition. AAT inhibits serine protease activity by forming a covalent 1:1 enzyme:inhibitor complex involving its reactive center MET-358 (Johnson and Travis, 1978). If SPAAT acts similarly then it too should form
25 such a stable enzyme:peptide complex. Thus, we tested the ability of SPAAT to bind to some physiologically relevant serine proteases and compared and contrasted this binding to that of AAT using our ELISA systems. As can be seen in Figure 3, both AAT and SPAAT bound to DFP-treated HNE as
30 well as cathepsin G.

BINDING COLLAGEN AND OTHER PROTEINS

35 Several additional observations indicate that SPAAT aggregates and/or binds to a larger molecular weight ECM "carrier" protein. First, immunohistochemical studies using antibodies against AAT reveal strong staining of the

adventitia of vessels in placenta villi as well as around the alveoli and small bronchi of the lung. Secondly, SDS-PAGE of placental SPAAT preparations reveal several high molecular weight protein bands in addition to the lower molecular weight SPAAT band. Moreover, amino acid analysis of our SPAAT preparations showed substantial amounts of hydroxyproline suggesting the presence of collagen chains with blocked N-termini since no collagen was detected in the sequencing studies. These results underscore the likelihood that in vivo SPAAT is bound to or deposited on biologically susceptible proteins and thus can play an important role in the protection of these proteins from inappropriate attack of serine proteases like HNE. Thus, SPAAT can be used in vivo to protect these proteins.

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SPAAT BINDS AN EXTRACELLULAR MATRIX PROTEIN WHICH INCREASES ACTIVITY

In further inhibition studies using chymotrypsin as the subject enzyme, we noted that the K_I (μM) for placental SPAAT is 0.92 while that for chemically synthesized SPAAT is 7.5. Thus, on a mole-for-mole basis, chemically synthesized SPAAT is an eight-fold less effective inhibitor of HNE than SPAAT isolated from placenta. These results demonstrate that association of the peptide with collagen in the placental material causes a conformational change more conducive to inhibitory capacity.

TABLE I

AMOUNT OF ELISA DETECTED PROTEIN
AT VARIOUS STEPS IN THE SPAAT EXTRACTION

Fraction	TOTAL DETECTED PROTEIN	
	AAT (mg)	HSA (mg)
1. 8M urea (Third extract)	≥ 22.14	ND
2. 8M urea + 2-ME (First extract)	≥ 36.38	ND
3. 8M urea + 2-ME (Second extract)	≥ 18.56	ND
4. Acid soluble supernatant	0.337	ND
5. Acid insoluble precipitate (Resuspended in 250 uls PBS)	≥ 0.0114	ND

AAT and HSA were detected antigenically by ELISA as described in the METHODS section of the text. Experimental values were calculated from a standard curve using appropriate commercially available antigen. Some fractions (≥) probably represent a minimum estimate of protein as even the lowest dilutions tested still exhibited maximum antibody binding. ND = none detected.

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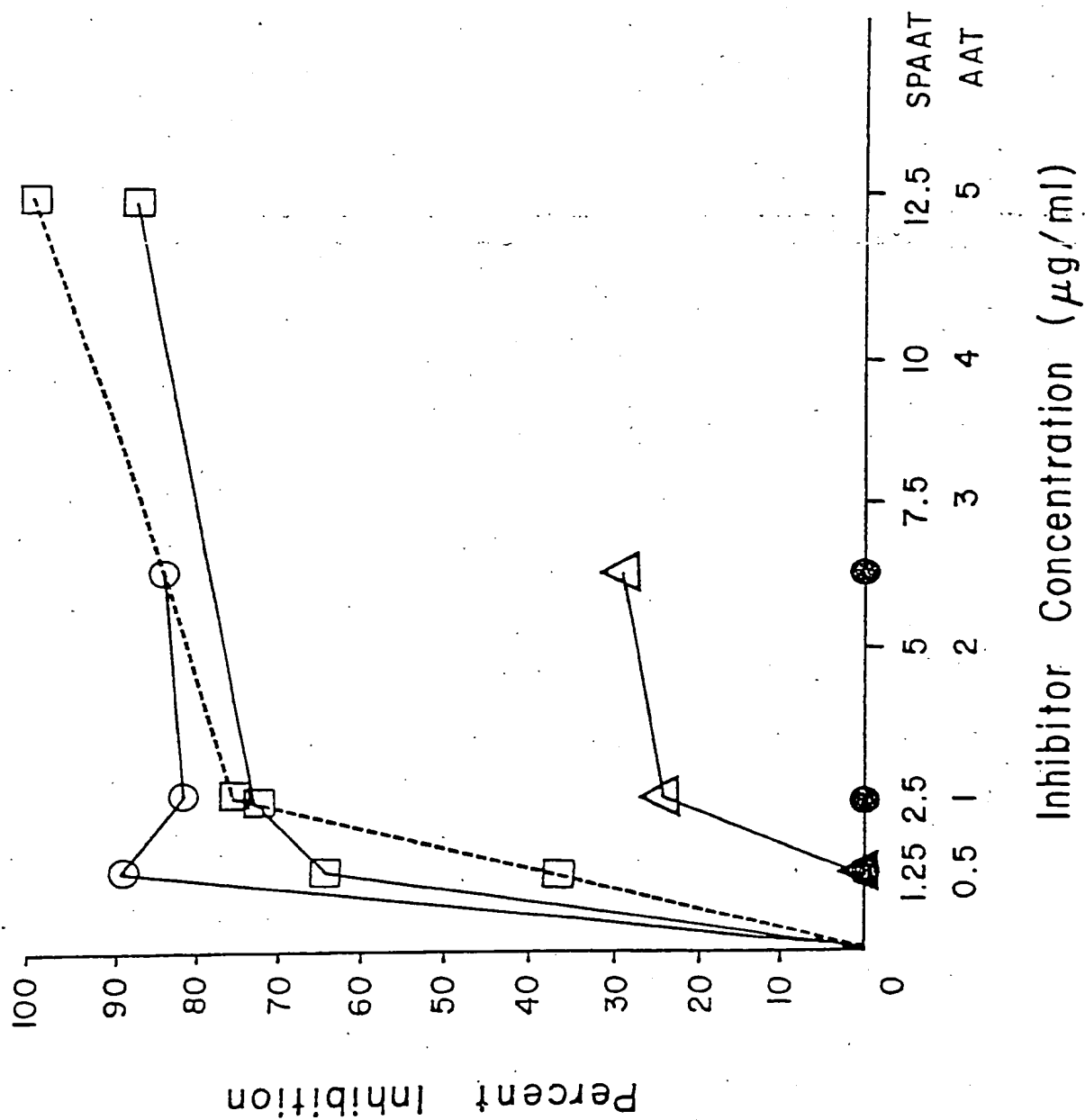
WHAT IS CLAIMED IS:

1. A compound comprising:
 - a) a polypeptide moiety having
 - (i) an identifying number of amino acids for SPAAT,
 - (ii) an elastase binding activity; and
 - b) an extracellular matrix protein bound to the polypeptide moiety.
2. The compound of Claim 1, wherein the extracellular matrix protein is a collagen.
3. The compound of Claim 2, wherein the collagen is type I collagen.
4. The compound of Claim 1, wherein the extracellular matrix protein is elastin.
5. The compound of Claim 1 in a pharmaceutically acceptable carrier.
6. A kit comprising:
 - a) a polypeptide moiety having
 - (i) an identifying number of amino acids for SPAAT,
 - (ii) an elastase binding activity; and
 - b) an extracellular matrix protein.
7. The kit of Claim 6, wherein the extracellular matrix protein is a collagen.
8. A method of inhibiting an elastase comprising contacting the elastase with a polypeptide moiety having:
 - a) an identifying number of amino acids for SPAAT;
 - b) a collagen binding activity; and
 - c) elastase binding activity.

9. The method of Claim 8, wherein the elastase is neutrophil elastase.
10. The method of Claim 8, wherein the polypeptide moiety
5 has the identical amino acid sequence of SPAAT.
11. The method of Claim 9, further comprising contacting the polypeptide moiety with an activity enhancing extracellular matrix protein prior to contacting the
10 elastase.
12. The method of Claim 11, wherein the extracellular matrix protein is a collagen.
- 15 13. The method of Claim 12, wherein the collagen is type I collagen.
14. The method of Claim 11, wherein the extracellular matrix protein is elastin.
20
15. A method of treating an aberrant condition associated with proteolysis by neutrophil elastase in a subject comprising administering to the subject a polypeptide moiety having:
25 a) an identifying number of amino acids for SPAAT;
 b) a collagen binding activity; and
 c) neutrophil elastase binding activity.
16. The method of Claim 15, wherein the aberrant
30 condition is selected from the group consisting of pulmonary emphysema and adult respiratory distress syndrome.

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FIGURE 1

	V	IV	III	II	I	
Residue #	350 +	+	+	+	+	360
AAT:	ALA - MET - PHE - LEU - GLU - ALA - ILE - PRO - MET - SER - ILE -					
SPAAT:	MET - PHE - LEU - GLU - ALA - ILE - PRO - MET - SER - ILE -					
						370
AAT:	PRO - PRO - GLU - VAL - LYS - PHE - ASN - LYS - PRO - PHE -					
SPAAT:	PRO - PRO - GLU - VAL - LYS - PHE - ASN - LYS - PRO - PHE -					
						380
AAT:	VAL - PHE - LEU - MET - ILE - GLU - GLN - ASN - THR - LYS -					
SPAAT:	VAL - PHE - LEU - MET - ILE - GLU - GLN - ASN - (THR) - LYS -					
						390
AAT:	SER - PRO - LEU - PHE - MET - GLY - LYS - VAL - VAL - ASN -					
SPAAT:	SER - PRO - LEU - PHE - MET - <u>LEU</u> LYS - VAL - VAL - <u>SER</u>					
						394
AAT:	PRO - THR - GLN - LYS - COOH					
SPAAT:						

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FIGURE 2

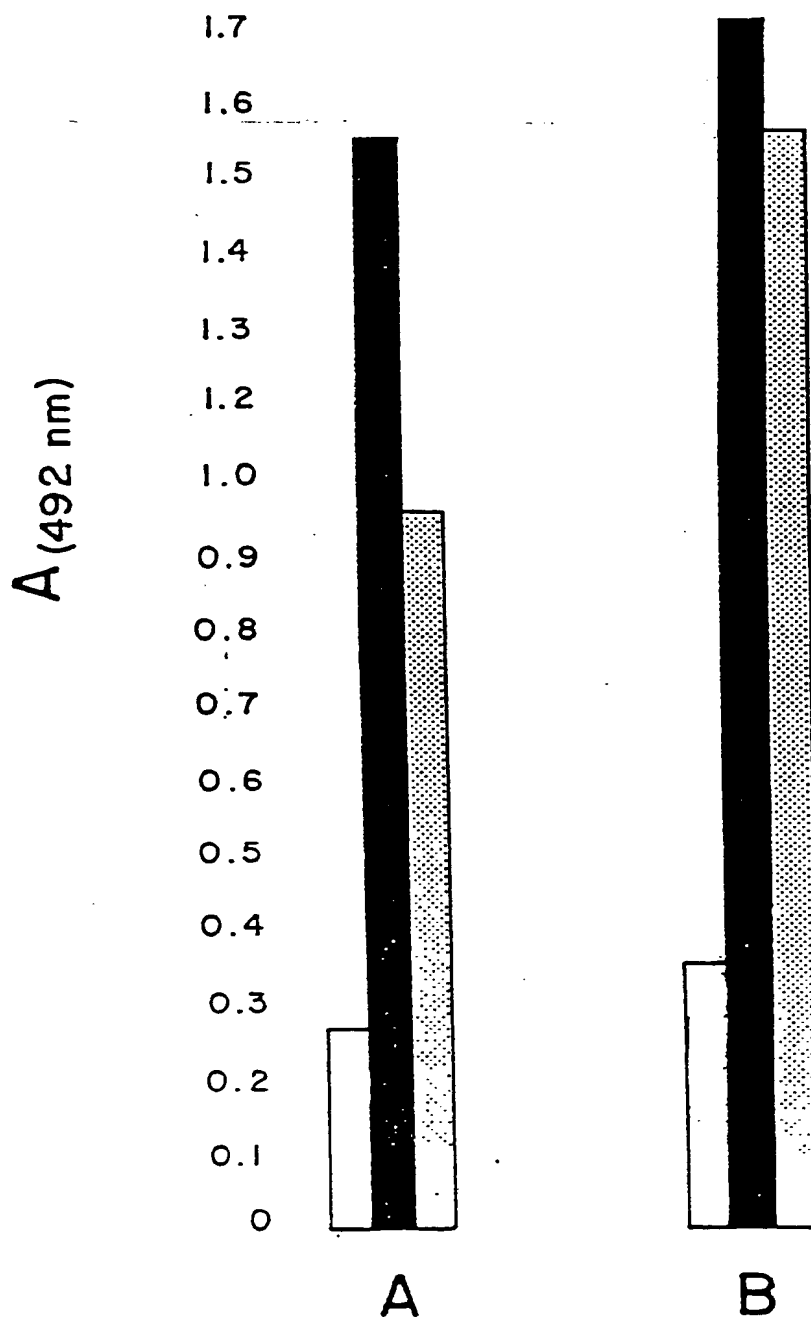
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FIGURE 3

□ = No Exogenous Protein Added

■ = + AAT

▨ = + SPAAT



INTERNATIONAL SEARCH REPORT

International application No.
PCT US92/03207

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00

US CL :514/12, 21; 525/54.1; 530/322, 323, 324, 362, 416, 417

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 21; 525/54.1; 530/322, 323, 324, 362, 416, 417

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Biochimica et Biophys. Acta, Volume 453, Issued 1976, Lo et al. "The Interaction of Alpha-1-Antitrypsin with Soluble and Sepharose-bound Elastase," pages 344-346, page 355, last para.	8-16
Y	Biochim. and Biophys. Res. Comm., Volume 86, Issued 1979, Satoh et al., "The Dual Nature of the Reaction Between Porcine Elastase and Human Plasma alpha-1-Proteinase Inhibitor," pages 130-137, see entire document.	8-16
Y	Birth, ed., Published 1986 by Academic Press, Inc., "Control of Elastin Synthesis: Molecular and Cellular Aspects," page 289, para. 3. See entire page.	11-14
Y	CA, A, 630,151 (Bloch and Oncson) 13 October 1961, see entire document.	11-14



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/LS92/03207

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,485,100 (Hochstrasser et al.) 27 November 1984, column 2, lines 50-53).	15-16
X	US, A, 4,496,689 (Mitra) 29 January 1985, see entire document.	

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